A farnesyl arabinoside as an enhancer of glucose transport in rat adipocytes from a soft coral, *Sinularia* sp.

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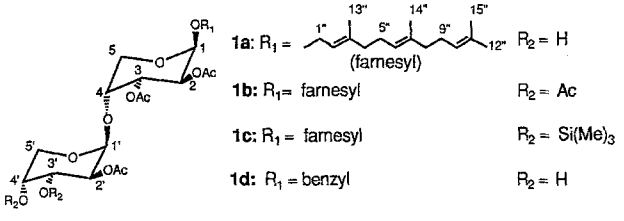
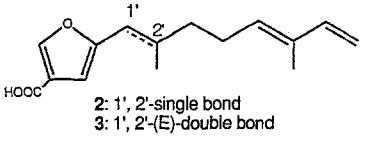
Abstract. Separation of a lipophilic extract of a soft coral, *Sinularia sp.*, assayed by enhancement of glucose transport in rat adipocytes, gave farnesyl 4-O-β-D-arabinopyranosyl-β-D-arabinopyranoside-2,2′,3-triacetate (**1a**) whose structure was determined by spectroscopy. Enhancers of glucose transport may be useful for the prevention and treatment of diabetic disorders.
Key words. Soft coral; *Sinularia* sp.; farnesyl arabinoside; glucose transport enhancer; diabetic complications.

Recently, we reported the structures of the aldose reductase inhibitors, pentabromopropen-2-yl-tribromo-and dibromoacetates. The inhibitors were isolated from the red alga, *Asparagopsis taxiformis*[1], by pharmacological screenings based upon the polyol pathway hypothesis[2] for the pathogenesis of diabetic complications such as retinopathy, cataract, neuropathy, angiopathy, and nephropathy. In most obese noninsulin-dependent diabetics whose endogenous insulin level is maintained at normal or slightly lowered levels, insulin administration is not always effective clinically. Noninsulin-dependent diabetes may be due to inadequate insulin effects on target cells, resulting in suppression of the glucose transport system and hyperglycemia. Therefore enhancers of glucose transport in insulin target organs may reduce the blood glucose level and be useful for the prevention and treatment of diabetic disorders[3,4].

In order to isolate natural enhancers from marine sources, we systematically tested extracts of marine organisms for enhancement of the 2-deoxyglucose (2-DG) uptake system in rat adipocytes[5], and found that the lipophilic extract of a soft coral, *Sinularia* sp. promoted 2-DG uptake. We report here the isolation, characterization, and 2-DG uptake activities of the active principles. *Sinularia* sp. (wet wt 1.3 kg) was collected by scuba diving in Okinawa in 1984 and thoroughly extracted with MeOH at room temperature. Assay-directed separation of the lipophilic extract by a combination of silica gel and reversed phase chromatography gave a novel active compound (**1a**, 24 mg)[6] together with two known active compounds 5-[2′,6′-dimethyl-(5′E)-octa-5′, 7′-dienyl]-3-furoic acid (**2**, 360 mg) and 5-[2′,6′-dimethyl-(1′E, 5E)-octa-1′,5′,7′-trienyl]-3-furoic acid (**3**, 240 mg)] previously isolated from *Sinularia capillosa*[7].

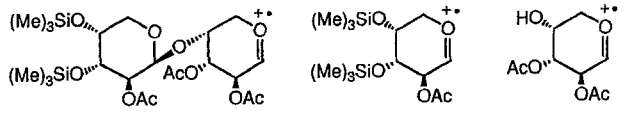
The compound (**1a**) was analyzed for $C_{31}H_{48}O_{12}$ by HRFABMS (M^+m/z612.3154, Δ + 0.8 mmu) and elementary analysis. The IR spectrum indicated the presence of hydroxyls [ν_{max}($CHCl_3$) 3550, 3450 cm^{-1}], which was confirmed by the formation of a pentaacetate (**1b**)[8] and a bis (trimethylsilyl)ether (**1c**)[9]. The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of four





olefinic methyls [δ_H 1.60 (br.s x 2), 1.675 (br. s.), 1.68 (d), δc 15.9 (q), 16.4 (q), 17.6 (q), 25.6 (g)], three trisubstituted double bonds [δ_H 5.09 (t), 5.10 (t), 5.29 (dd), δc 119.0 (d), 123.2 (d), 123.7 (d), 130.7 (s), 134.8 (s), 140.9 (s)]. a methylene [δ_H 4.03 (dd), 4.15 (dd), δ_C 63.9 (t)], and two ethylenes (δ_H 2.05-2.15), compatible with a farnesol moiety. The other NMR features indicated the presence of two sets of arabinopyranose moiety, three of whose hydroxyls were acetylated [δ_H 2.06 (s), 2.08 (s), 2.15 (s)] and located on C-2 [δ_H 5.21 (dd)],-2′[δ_H 4.90 (dd)] and -3 [δ_H 5.28 (dd)]. The farnesyl group was connected to C-1 through a β-glycosidic linkage in the $^1C_4$ conformation of the arabinopyranose ring, supported by the coupling constant ($J_{1,2}$ = 3.5, $J_{2,3}$ = 10.5 Hz)[10] and the N.O.E. between H-1 and -1″. The absence of any other saccharose in **1a** was also suggested by the exclusive formation of methyl arabinoside on alkaline hydrolysis followed by treatment with 6N-HCl/MeOH at 100 °C[11].

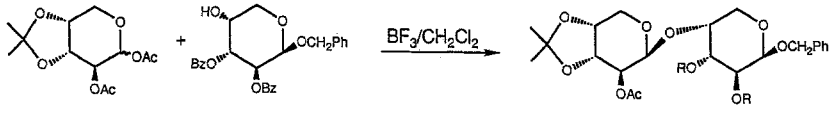
In addition to the EIMS of **1a** and **1b**, the compound (**1c**) showed some diagnostic fragment ions (**I, II, III**) at m/z 535 ($C_{22}H_{39}O_{11}Si_2$), 319 ($C_{13}H_{27}O_5Si_2$), and 217



^1H and ^{13}C NMR data of **1a**[a] and **1d**[b] in $CDCl_3$

1a No.	^{13}C (mult.)	^1H (mult., J, Hz)	**1d** No.	^{13}C (mult.)	^1H (mult., J, Hz)
1	94.6 (d)	5.06 (d, 3.5)	1	95.2 (d)	5.10 (d, 3.7)
2	68.2 (d)	5.21 (dd, 3.5, 10.5)	2	68.3 (d)	5.22 (dd, 3.7, 11)
3	68.4 (d)	5.28 (dd, 3.5, 10.5)	3	68.6 (d)	5.33 (dd, 3.3, 11)
4	69.0 (d)	4.06 (ddd, 1, 2.5, 3.5)	4	69.3 (d)	4.0–4.2 (m)
5	59.3 (t)	3.54 (dd, 2.5, 11.5)	5	59.8 (t)	3.55 (dd, 2, 12.5)
		3.82 (dd, 1, 11.5)			3.84 (dd, 1.8, 12.5)
1′	94.9 (d)	5.15 (d, 3.9)	1′	95.5 (d)	5.16 (d, 3.7)
2′	71.9 (d)	4.90 (dd, 3.9, 10)	2′	71.9 (d)	4.89 (dd, 3.7, 10.2)
3′	67.2 (d)	4.14 (dd, 3.5, 10)	3′	67.3 (d)	4.0–4.2 (m)
4′	73.9 (d)	4.04 (td, 2, 3.5)	4′	73.9 (d)	4.0–4.2 (m)
5′	62.1 (t)	3.72 (dd, 2, 12)	5′	62.5 (d)	3.72 (br. d, 12.4)
		4.06 (dd, 2, 12)			4.0–4.2 (m)
1″	63.9 (t)	4.03 (dd, 7.5, 12)	CH_2	69.5 (t)	4.51 (d, 12.5)
		4.15 (dd, 7, 12)			4.74 (d, 12.5)
2″	119.0 (d)	5.29 (dd, 7, 7.5)	Ph	127.5 (dx2)	7.2–7.5 (m)
3″	140.9 (s)			127.7 (d)	7.2–7.5 (m)
4″	39.52 (t)	2.05–2.15 (m)		128.3 (dx2)	7.2–7.5 (m)
5″	22.6 (t)	2.05–2.15 (m)		137.0 (s)	
6″	123.2 (d)	5.10 (t,7)	Ac	169.9 (s)	
7″	134.8 (s)			170.2 (s)	
8″	39.46 (t)	2.05–2.15 (m)		171.4 (s)	
9″	26.3 (t)	2.05–2.15 (m)		20.8 (q)	2.04 (s)
10″	123.7 (d)	5.09 (br.t, 7)		20.9 (qx2)	2.06 (s)
11″	130.7 (s)				2.15 (s)
12″	25.6 (q)	1.68 (d, 1.5)			
13″	16.4 (q)	1.675 (br.s)			
14″	15.9 (q)	1.60 (br.s)			
15″	17.6 (q)	1.60 (br.s)			
Ac	169.2 (s)				
	169.6 (s)				
	170.8 (s)				
	20.8 (qx3)	2.06 (s)			
		2.08 (s)			
		2.15 (s)			

[a] The ^1H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively.
[b] The ^1H and ^{13}C NMR were recorded at 270 and 25.5 MHz, respectively.



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1e: R=benzoyl
1f: R=Ac

($C_9H_{13}O_6$) respectively in the EIMS. The fragment ion **II** showed that two free hydroxyls were vicinally located at C-3′ and -4′. The fragment ion **III** indicated that another β-glycosidic (bond $J_{1',2'} = 3.9$ Hz, $J_{2',3'} = 10$ Hz) must be formed between C-4 and -1′ in the $^1C_4$ arabinopyranose ring, supported by the N.O.E. between H-4 and -1′.

With a strong negative optical rotation, **1a** could be consequently depicted as farnesyl 4-O-β-D-arabinopyranosyl-β-D-arabinopyranoside-2,2′,3-triacetate. This structure was strongly supported by comparison of the ^1H and ^{13}C NMR data and optical rotation between **1a** and a model compound (**1d**)[12] unambiguously prepared from D-arabinose via several steps; Benzyl 2,3-dibenzoyl-β-D-arabinopyranoside (**5**)[13] underwent glycosidation[14] with acetyl 2-acetyl-3,4-O-isopropylidene-D-arabinopyranoside (**4**)[13] in the presence of BF_3 etherate in dry CH_2Cl_2 to give a desired β-anomer (**1e**), albeit in low yield, which was converted to **1d** through debenzoylation, acetylation, and acetonide cleavage successively.

The compound (**1a**) enhanced 2-DG uptake with a 2-DG uptake index of 1.57 (enhancing ratio to control). Two known 3-furoic acids (**2,3**) also had an enhancing effect with indices of 1.67 and 1.33 respectively, but did not lower the blood glucose level in vivo. The tests in vivo of **1a** are underway.

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6 **1a**:oil. $[\alpha]_D - 196°$ (c = 0.75, $CHCl_3$). IR [ν_{max} cm^{-1}($CHCl_3$)]3550, 3450, 1735, 1385, 1240, 1180, 1060. Elementary analysis calcd. for $C_{31}H_{48}O_{12}$ C, 60.78; H, 7.84, found C, 60. 64; H, 7.97.

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8 **1b**: oil. IR[ν_{max} cm^{-1}($CHCl_3$)] 1740, 1380, 1250, 1160. ^1H NMR [δ($CDCl_3$)] 1.56 (3H,s), 1.60 (3H, s), 1.65 (3H, s), 1.68 (3H, d, J = 1.5 Hz), 1.95-2.15 (8H, m), 2.03 (3H, s), 2.08 (6H, s), 2.10 (3H, s), 2.14 (3H, s), 3.51 (1H, dd, J = 2.5, 12.7 Hz), 3.65 (1H, dd, J = 1.9, 13.2 Hz), 3.81 (1H, d, J = 12.7 Hz), 4.02 (1H, dd, J = 7.3 ,12 Hz), 4.04 (1H, m), 4.13 (1H, d, J = 12 Hz), 4.14 (1H, dd, 7, 12 Hz), 5.05-5.4 (10H, m). Elementary analysis Calcd. for $C_{35}H_{52}O_{14}$ C, 60. 34; H, 7.47. Found C, 60.52; H, 7.40.
9 **1c** was prepared on treatment of **1a** with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine.
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12 **1d**: oil. $[\alpha]_D$ −204.2° (c = 1.78, $CHCl_3$). IR [ν_{max} cm^{-1} ($CHCl_3$)] 3560, 3550, 1640, 1500, 1370, 1210, 1140, 1060, 1015. EIMS (m/z) 259, 235, 217, 175, 157, 91.
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Trail-following responses of *Leptogenys diminuta* to stereoisomers of 4-methyl-3-heptanol [1]

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Abstract. Behavioral tests carried out with the four stereoisomers of 4-methyl-3-heptanol revealed that *Leptogenys diminuta* ants respond specifically only to the (3*R*, 4*S*)-isomer.
Key words. Trail pheromone; 4-methyl-3-heptanol; *Leptogenys diminuta*; Formicidae; Ponerinae; ant; chirality.

Leptogenys diminuta Smith, a common ponerine ant found in southeastern Asia, lays orientation trails by depositing the contents of the poison gland secretion. Recently we identified the trail pheromone of *L. diminuta* as (3*R*, 4*S*)-4-methyl-3-heptanol [2]. Precise preception of pheromonal signals plays a crucial role in the life of many insects. Although many models have been proposed, the olfactory perception of pheromonal messages at the molecular level is still poorly understood. Studies based on structure-activity relationships assist us to understand how odorants interact with the receptors. As very pure synthetic stereoisomers of 4-methyl-3-heptanol became available [3], we were able to conduct stereochemistry-pheromone activity studies on the workers of *L. diminuta* in order to verify whether the non-natural isomers are neutral or show some biological acitivity.

Materials and methods

Chemicals. Samples of (3*RS*, 4*R*)-4-methyl-3-heptanol and (3*RS* ,4*S*)-4-methyl-3-heptanol were synthesized as diastereomeric mixtures by Frighetto in our laboratory [4]. Four stereoisomers of 4-methyl-3-heptanol were a gift of Prof. Matteson (Washington State University). Purity of the chemicals and concentrations of the solutions were determined by capillary gas chromatography.

Ants. Several colonies of *L. diminuta* were collected from sites near Ulu Gombak Experimental Station in Malaysia. The colonies were transferred to Frankfurt and maintained on a diet of live arthropods (nymphs of *Blaberus discoidalis* (Blattoidea) and *Acheta domestica* (Saltatoria), and larvae of *Tenebrio molitor* (Coleoptera)).

Quantification. Individual poison glands excised from worker ants were analyzed by capillary gas chromatography as described previously [2]. A solution of authentic 4-methyl-3-heptanol was used as an external standard for quantification.

Trail-following tests. The trails were drawn with a lead pencil on a white piece of cardboard and the test chemicals were applied, as ethanol solutions, by disposable glass capillary tubes (10 µl, Brand). A standard trail test consisting of two intercrossing S-shaped lines (each 20 cm) was used when two substances were being tested for relative activity. One of the lines was streaked with one test chemical and the other with another test chemical or solvent as control. The test was repeated 15 times and the choice response of the first worker that followed the trail was noted. Samples prepared by Frighetto [4] containing (3*RS*, 4*R*)-4-methyl-3-heptanol and (3*RS*, 4*S*)-4-methyl-3-heptanol were tested in this way at 50 pg/cm level.

In order to determine the trail-following threshold of *L. diminuta*, different amounts of (3*RS*, 4*S*)-4-methyl-3-heptanol were applied to a trail (100–0.01 pg/cm) and tested against a control trail streaked with solvent ethanol. The response of each ant that followed the 20-cm trail to the end was considered positive; incomplete or